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SCHOOL OF MEDICINE
Department of Microbiology
and Immunology

SAN FRANCISCO, CALIFORNIA 94143

July 5, 1984

Professor Kalman Perk
Director
School of Veterinary Medicine
The Hebrew University of Jerusalem
Jerusalem, ISRAEL

Dear Dr. Perk:

I was very pleased to learn of your interest in a collaborative effort to identify the target gene for insertional mutagenesis in retrovirus-induced pulmonary tumors. An incoming post-doctoral fellow, Dr. Paul Bates, has expressed a strong interest in this project and will be proposing to work on it in his applications for post-doctoral fellowships. Since he will not arrive here until late in 1984 or early in 1985, there is time for us to consider the best way to organize the project.

We currently entertain three ways to look for activation of oncogenes by proviral insertions. (i) Known oncogenes can be tested for rearrangements caused by insertion of proviruses, using the Southern DNA transfer procedure to analyze DNA from tumor and normal tissue with the 20 or so available probes for oncogenes. For such tests, we like to have at least a gram of relatively homogeneous tumor tissue and similar amounts of a normal organ from the same animal. The samples need to be quick-frozen to avoid extensive degradation of nucleic acid. A gram of tissue will usually yield close to a mg of DNA, sufficient for multiple analyses, plus DNA cloning if called for. Obviously, when possible, it is good to have larger samples to permit multiple preparations of DNA and for tests of RNA and (if called for) protein at later stages. (ii) If the first approach fails, we can seek novel oncogenes that might be provirally activated by cloning the cell DNA that flanks proviral DNA in tumors carrying a single new provirus. The flanking DNA is then used as a probe to seek evidence that the same integration site is occupied in other tumors (see reprint by Nusse and me). For these experiments we need a way to make probe specific for your virus isolate (assuming its genome is not cross reactive with other mammalian retroviruses). So we'd need enough virus stock to make labeled cDNA, either in an endogenous polymerase reaction or in a reaction templated with viral RNA purified from your virus. This would require a minimum of 100 ug of viral protein (with an anticipated yield of 1-2 ug of viral RNA). Can you give us some idea of the titre of your stocks and the amount of virus that could be made available? Once some probe was in hand, we would proceed to clone a provirus from some suitable tumor or infected cells in culture, using enzymes to be chosen after preliminary restriction mapping. The clone would then serve

as a probe for future experiments with tumors. It would probably be necessary to have samples from at least 10-20 tumors to have a reasonable chance of finding one with a single provirus and to have a bank of DNA's against which to test putative common integration sites. (iii) The third approach involves a search for hybrid (viral-host) transcripts indicative of promotion within an LTR and transcription of flanking host (oncogene) DNA. We have made successful use of this method recently in an avian nephroblastoma that proves to have an LTR-activated c-Ha-ras gene, although methods (i) and (ii) failed to detect this mutation. Here we would need tumors that were frozen with sufficient dispatch to yield intact RNA.

At this stage, it would be useful to have some notion of the abundance of materials at your disposal: tumors (size and numbers), control tissues, and virus stocks. I look forward to hearing from you.

With best regards,

Harold E. Varmus, M.D.
American Cancer Society
Professor of Molecular Virology

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Enclosures